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Biosurfactants in Electrospinning

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Biosurfactants in Electrospinning

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Honors Research Project

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Executive Summary

Electrospinning is a simple and versatile process for producing nano to micro size fibers. Three main parameters affect the electrospinning process. These parameters are the applied voltage, polymer flow rate, and capillary-collector distance. The addition of surfactants to the electrospun solution can improve fiber morphology. Crocin is a natural water soluble surfactant that can be mixed with poly(ethylene oxide), PEO, to improve the electrospinning of PEO. The study focused on first establishing the electrospinning parameters, behavior, and fiber morphology of PEO under varying voltages, polymer flow rates, and capillary-collector distance. Then Crocin was added to the PEO and the fiber diameter and morphology was compared to PEO electrospun at the same conditions. The viscosity of the solution was determined using a viscometer and the surface tension was determined using the pendant drop method. Fiber morphology was examined using SEM and fiber diameters were determined using ImageJ to measure the fibers in the SEM images.

The PEO case study showed that increasing concentrations of PEO from 4% to 6% caused increased fiber diameters. Changing the applied voltage caused an initial increase in fiber diameter for 4% and 5% PEO when the applied voltage was changed from 15 kV to 20 kV, but little change in fiber diameter when increased from 20 kV to 25 kV. For 6% PEO, the fiber diameter decreased from 15 kV to 20 kV but increased from 20 kV to 25 kV. Increasing concentrations of PEO also led to improved fiber morphology. Fibers were smoother and had less defects such as beads at higher concentrations.

Adding Crocin to the solution reduced the surface tension of PEO when compared to the PEO without Crocin. The addition of Crocin also reduced the solution viscosity. When electrospinning the PEO and Crocin solutions, the results showed that the Crocin reduced the fiber diameter by 31%±1% for the sample spun at 20 kV and 37%±0.5% for the sample spun at 25 kV. The Crocin also improved the fiber morphology by producing smoother fibers.

Through this work, I learned more about the research process. Research can be open ended and unstructured. The researcher must define constraints for the project in terms of funding, current research, and goals for the project. It is very unlike a classroom setup where the steps and outcomes are clearly defined. A good portion of the time is spent doing background research to determine the state of the topic and then determining what the next steps are or determining areas where questions or uncertainties may lie. This process helped to strengthen my

research abilities. The successful design of experiments and interpretation of the results was also an important learning experience of this project. I worked fairly independently on this project with the help of the graduate student I worked with. In writing the report and performing the study, I realized that electrospinning and its applications are still in its infancy. The applications for it and the modifications of the electrospun fibers has many applications that are just being discovered and studied.

Next steps would include a more expansive study of the PEO and Crocin role which would include more electrospinning characterization and looking into whether the Crocin is located in or on the fiber surface. If the Crocin is on the fiber surface, the Crocin may be able to be modified to change the surface chemistry of the fiber for drug loading or other applications.

Introduction

Electrospinning is a simple and versatile process that allows for the production of nano to micro size fibers from a variety of natural and synthetic polymers (Wang et al., 2008). The typical electrospinning set up consists of a high voltage supply, a grounded collector plate, and a spinneret as seen in **Figure 1** (Li and Xia, 2004). As an electric charge is applied to the solution, the charges shift to the surface of the polymer at the tip of the capillary which is held in place by the solution's surface tension. At the tip, electrostatic repulsions between charges cause the drop at the end of the capillary to deform into a Taylor cone. At a threshold voltage, the electrostatic repulsions overcome the surface tension of the solution and a fiber is ejected from the apex of the Taylor cone (Wang et al., 2008). The fibers undergo a chaotic bending and whipping path as it travels from the spinneret to the grounded collector allowing the solvent to evaporate and the fiber to be stretched. The chaotic bending and whipping can reduce the fiber diameter from micrometer size to nanometer size. The fibers are deposited on a stationary collector plate as a randomly oriented, non-woven fiber mat. A common problem in electrospun fibers is the presence of beads which can be reduced by the addition of a surfactant (Li and Xia, 2004), (Ziani et al., 2011).

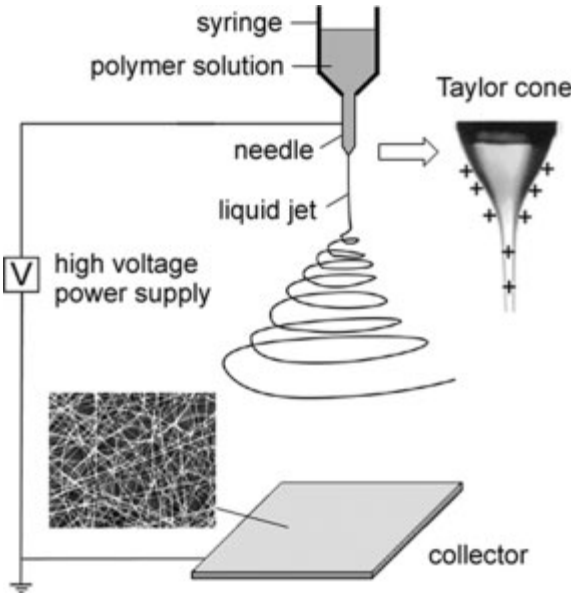

Figure 1. Typical electrospinning setup.

The process of electrospinning as a viable technique began in the early 1930's with Formhals. In 1934, he patented his first process for producing fibers using a moveable fiber collecting drum to collect the ejected fibers. His first patent had several disadvantages. The distance between the collector and the spinneret was too short resulting in fibers that were not completely dry being collected. Formhals continued to improve on his design and patent other methods for producing fibers. (Subbiah et al., 2005)

In the 1960's Taylor studied the jet forming process. Taylor studied the shape of the droplet at the tip of the capillary during electrospinning and discovered the Taylor cone and the process underlying the ejection of the fiber from the capillary. With the capability of more advanced testing such as scanning electron microscopy, differential scanning calorimetry, and wide-angle X-ray diffraction, researchers focused more on the morphology of the fibers through the 1970's and 80's. (Subbiah et al., 2005)

In the late 1990's and early 2000's, a renewed interest in electrospinning took place due to the realized application potential for the fibers (Subbiah et al., 2005). The research was led in part by Doshi and Reneker who studied the characteristics of polyethylene oxide (PEO) by varying solution and operational parameters. Interest and research continues to grow exponentially as new techniques and applications are realized for electrospun fibers (Li and Xia, 2004).

The potential applications for electrospun fibers extend from some of the peculiar properties nanofibers can have including high strength, high surface energy, exceptional thermal conductivity, and a large surface to volume ratio. These properties make the fibers useful for in a variety of applications in different areas including microfluid channels, sensors, catalysis, energy storage, environmental engineering and tissue engineering. The following work will focus on the use of a biocompatible polymer and a natural surfactant in electrospinning for applications in biomedical applications.

Background

The processing parameters used to produce the electrospun fibers can affect the fiber formation and structure. Three major parameters affect the electrospinning process. These factors are, in order of relative importance to fiber morphology and diameter, applied voltage, polymer, flow rate, and capillary-collector distance.

The strength of the applied voltage has an important role in the changing the fiber diameter and in the formation of beads. Too low of a voltage can lead to bead defects or even prevent fibers from forming while too high of a voltage can also cause the formation of beads. With increasing voltage the shape and location of the Taylor cone changes. When the Taylor cone is first formed, it forms at the tip of the pendant drop. As the voltage is increased, the pendant drop shrinks and eventually the Taylor cone forms at the tip of the capillary as seen in **Figure 2**. Increasing the voltage beyond this point causes the jet to originate from within the capillary which has been associated with increased bead defects. The fiber diameter also changes with increasing voltage. Initially, increasing the voltage will decrease the fiber diameter. However, after a certain point, the fiber diameter will start to increase (Sill and von Recum, 2008).

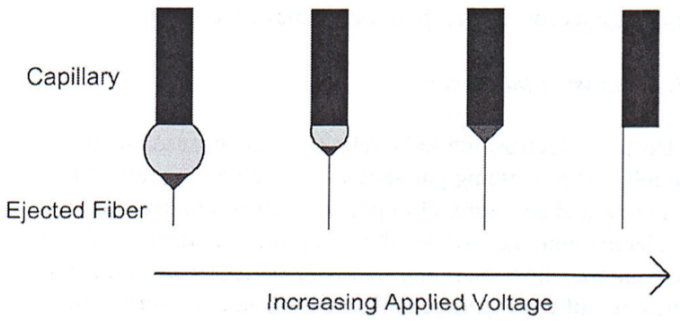

Figure 2. Shape of pendant drop with increasing voltage. (Sill and von Recum, 2008)

The flow rate of the solution can influence the fiber diameter and shape. An increase in flow rate flow rate is associated with an increase in fiber diameter. If the flow rate is not high enough, the Taylor cone cannot be maintained at the tip of the capillary, which can be associated with bead

defects as the jet begins to originate from within the capillary as seen in **Figure 2**

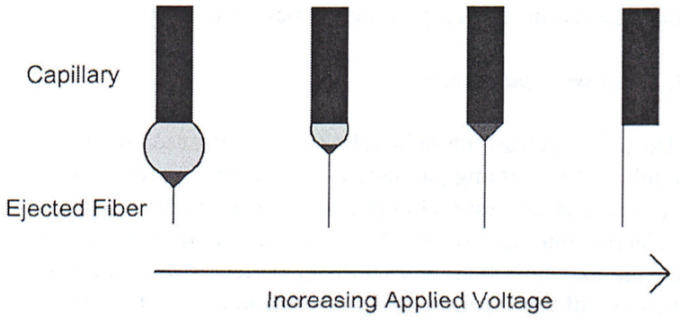

Figure 2. Flow rates that are too high can lead to bead defects and flat, ribbon fibers since the fibers do not have enough time to dry before reaching the collector (Sill and von Recum, 2008).

The capillary-collector distance influences the fiber diameter and fiber morphology. Increasing the capillary-collector distance causes a decrease in fiber diameter. If the distance is too short, inadequate drying time may lead to beaded or ribbon fibers. The capillary-collector distance can also be a determining factor between electrospinning and electrospraying (Sill and von Recum, 2008).

Surfactants are amphilic molecules that act as surface active agents which lowers the surface tension of the solution and can influence the electrospinning process (Kriegel et al., 2009). Crocin is a natural surfactant and a yellow pigment that is extracted from gardenia yellow and saffron (Tamaddonfard and Hamzeh-Gooshchi, 2010). Crocin is one of the few highly water-soluble natural unsaturated conjugated sugars abundantly available. It has also been shown to have antioxidant and cancer cell growth inhibiting properties (Naess et al., 2006). A natural surfactant paired with a biocompatible polymer could be useful in biomedical applications.

Electrospinning provides the ability to engineer scaffolds that mimic the native extracellular matrix (ECM), which helps to support and regulate cells among many other tasks, within the body (Nagiah et al., 2012). Electrospun fiber mats can be created with micro to nanoscale topography, high porosity, a high surface to volume ratio, and an ability to incorporate drugs such as antibiotics and anticancer agents which allows control over the scaffold properties and allows the scaffold to mimic the ECM (Sill and von Recum, 2008). The scaffolds have uses in tissue engineering in regenerating lost or damaged tissue and in drug delivery.

This study examines the use of Crocin to improve the electrospinning of PEO, a biocompatible synthetic polymer that has been approved for internal use in pharmaceutical and food. PEO was to be spun with and without Crocin at varying voltages, flow rates, and capillary-collector distances to analyze the effect Crocin had on the electrospun fibers morphology and diameter. Solution properties including surface tension and viscosity were measured.

Experimental Methods

PEO was purchased from Scientific Polymer with a molecular weight (MW) of 400,000 grams per mole. PEO solutions of 4%, 5%, and 6%, weight percent, were prepared using deionized (DI) water. Solutions were dissolved by mixing at room temperature with a stir bar and stir plate overnight. Crocin was purchased from Trade TCI. Crocin solutions were prepared using DI water and mixing by gentle shaking of container.

Fibers were obtained by electrospinning solutions of PEO and Crocin in DI Water. The lab setup used is pictured below in **Figure 3**. A syringe pump, New Era Pump Systems Inc., was used to control flow rates. PVC piping was used to control gap distance from the charged needle to the grounded collector plate. The voltage source, Gamma High Voltage Research, was used to adjust the supplied voltage to the system. Only one parameter, voltage, gap distance, or flow rate, was altered at one time during testing.

Solutions were characterized by their viscosity, surface tension, and polymer concentration. Viscosity was tested using a Brookfield viscometer in Dr. Chuang's lab. Each sample was tested at a controlled temperature of 20°C, with spindle CPE 52 which has a diameter of 2.5 cm, and at 5 different shear rates (RPM's) using 1.5 to 2 ml of solution. Measurements were taken in triplicate to ensure accuracy. Surface tension was tested using a Drop Shape Analyzer (DSA), Easy Drop model FM40, in Dr. Chase's lab. Samples were tested by forming the largest stable droplet at the tip of the syringe and analyzed by software to calculate the surface tension using the pendant drop method. Trials were repeated five times and averaged by the software to determine the surface tension.

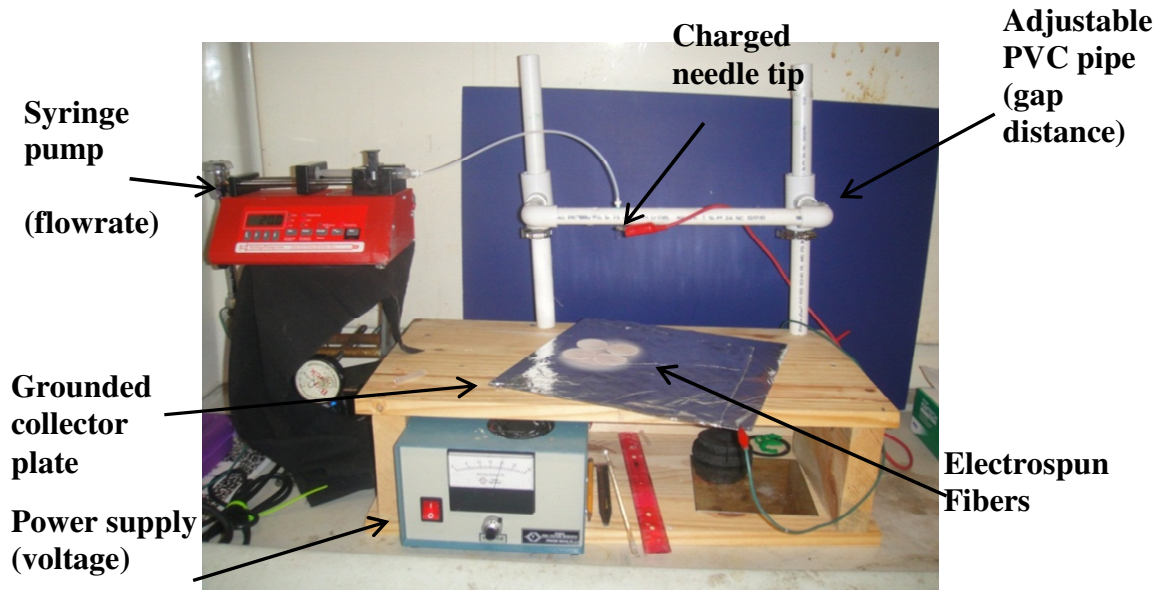

Figure 3. Electrospinning setup.

The experiment was designed to first electrospin PEO at various conditions to establish the behavior of the electrospun PEO and help determine conditions to spin the PEO and Crocin solutions. The flow rate, gap distance, and voltage were each varied while holding the others constant. Once the PEO fibers were electrospun, a similar procedure was used for the PEO and Crocin fibers to fiber morphology and diameter. Fiber morphology was examined using scanning electron microscopy (SEM) and fiber diameters were determined using ImageJ to measure the fibers in the SEM images.

Data and Results

PEO was first spun at concentration of 4%, 5%, and 6% at flow rates of 10 μL/min, 15 μL/min, and 20 μL/min, voltages of 15 kV, 20 kV, and 25 kV, and capillary-collector distances of 10 cm, 15 cm, and 20 cm. No fibers were formed when electrospinning at a capillary-collector distance of 10 cm and poor quality fibers were formed at a gap distance of 20 cm. Poor quality fibers were formed using flow rates of 10 μL/min and 20 μL/min. **Figure 4** shows the fiber diameters of 4%, 5%, and 6% PEO using 15 cm, 20 kV, and 20 μL/min. Higher concentrations of PEO showed a higher fiber diameter. All conditions had a range of fiber diameters as indicated by the error bars which represent one standard deviation. **Figure 5** shows example images of the measured fibers from **Figure 4**. The lower concentrations of PEO had increased fiber defects such as beads.

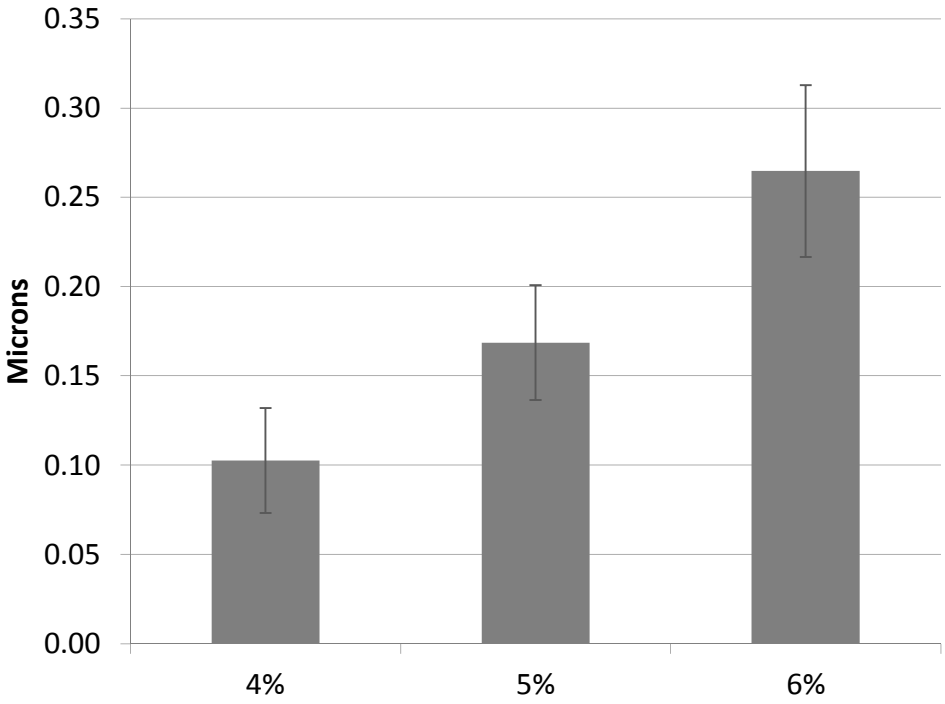

Figure 4. PEO concentrations of 4%, 5%, and 6% spun at 15 cm, 20 kV, and 20 μL/min.

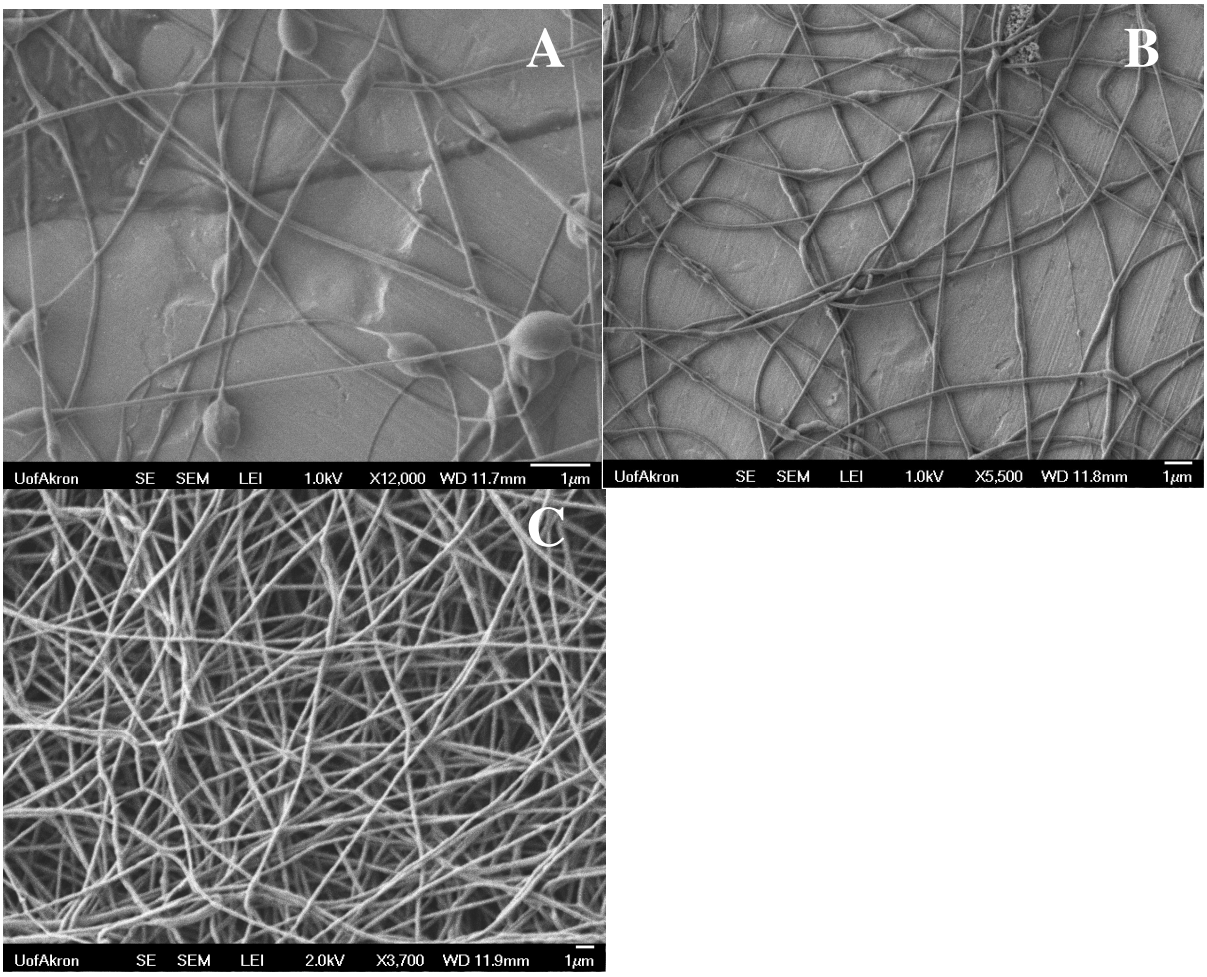

Figure 5. A. 4% PEO at 15 cm, 20 kV, and 20 μL/min. B. 5% PEO at 15 cm, 20 kV, and 20 μL/min. C. 6% PEO at 15 cm, 20 kV, and 20 μL/min.

Using the base conditions of 15 cm, 20 kV, and 20 μL/min, the voltage was varied for the three different concentrations of PEO as seen in **Figure 6**. Higher concentrations of PEO led to larger fiber diameters at all conditions. Increasing the voltage for the 4% PEO led to an initial increase in fiber diamter from 15 kV to 20 kV, but the change from 20 kV to 25 kV produced no real change in fiber diameter. Similar results were seen with the 5% PEO. The fiber diameter of 6% PEO decreased from 15 kV to 20 kV, but fiber diameter increased from 15 kV and 20 kV to 25 kV. All conditions had a range of fiber diameters as indicated by the error bars which represent one standard deviation. **Figure 7**, **Figure 8**, and **Figure 9** show example images of the measured fibers from **Figure 6**. The lower concentrations of PEO had increased fiber defects such as beads at all voltages.

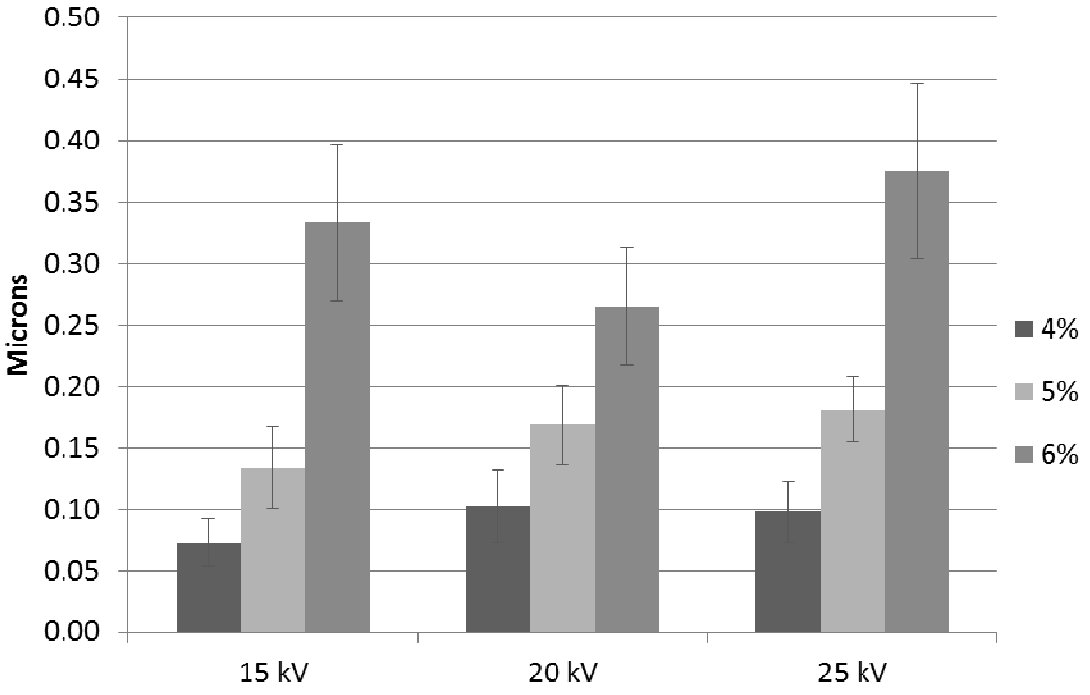

Figure 6. Varying concentrations of PEO at varying voltages and the fiber diameter of each.

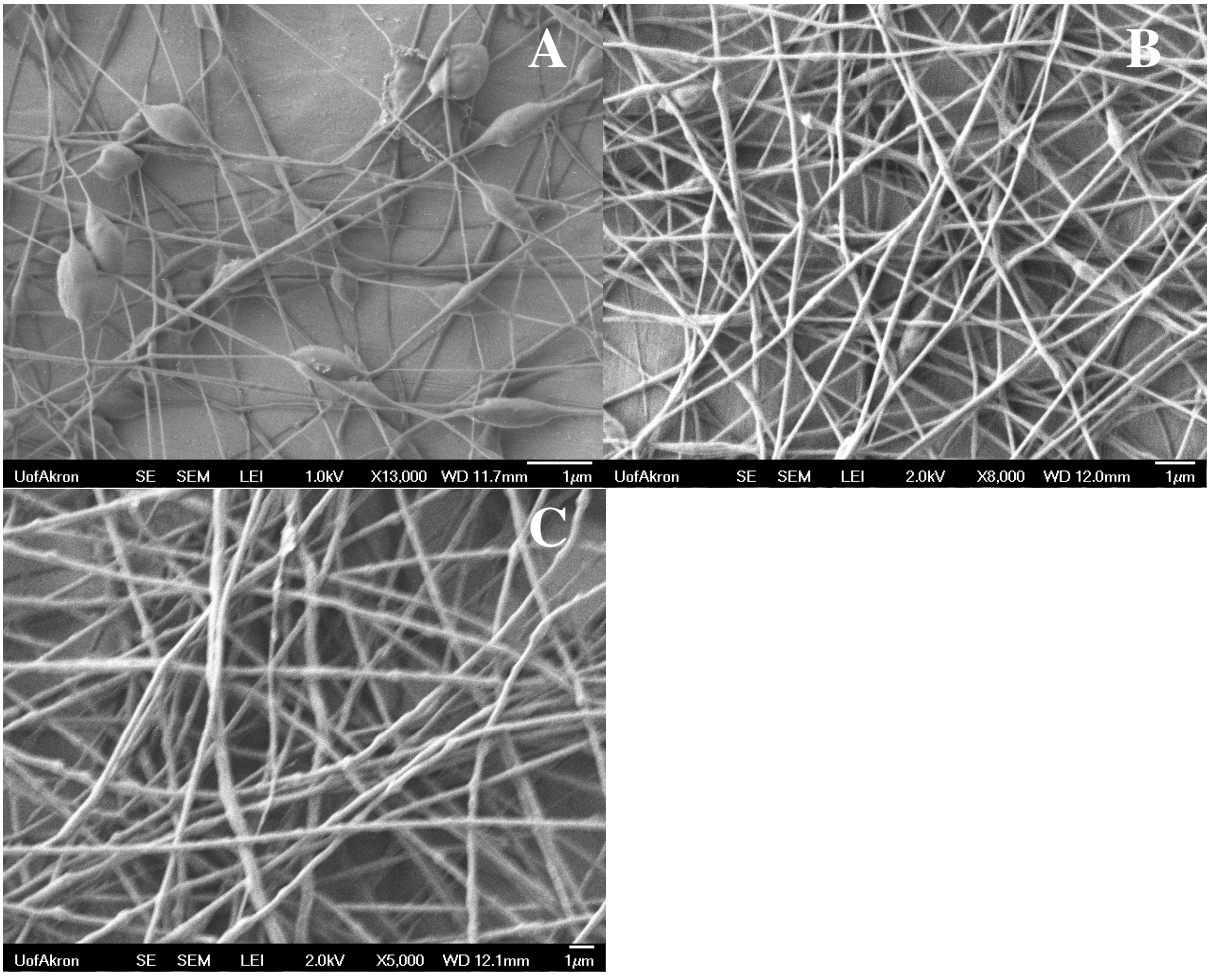

Figure 7. A. 4% PEO at 15 cm, 15 kV, and 20 μL/min. B. 5% PEO at 15 cm, 15 kV, and 20 μL/min. C. 6% PEO at 15 cm, 15 kV, and 20 μL/min.

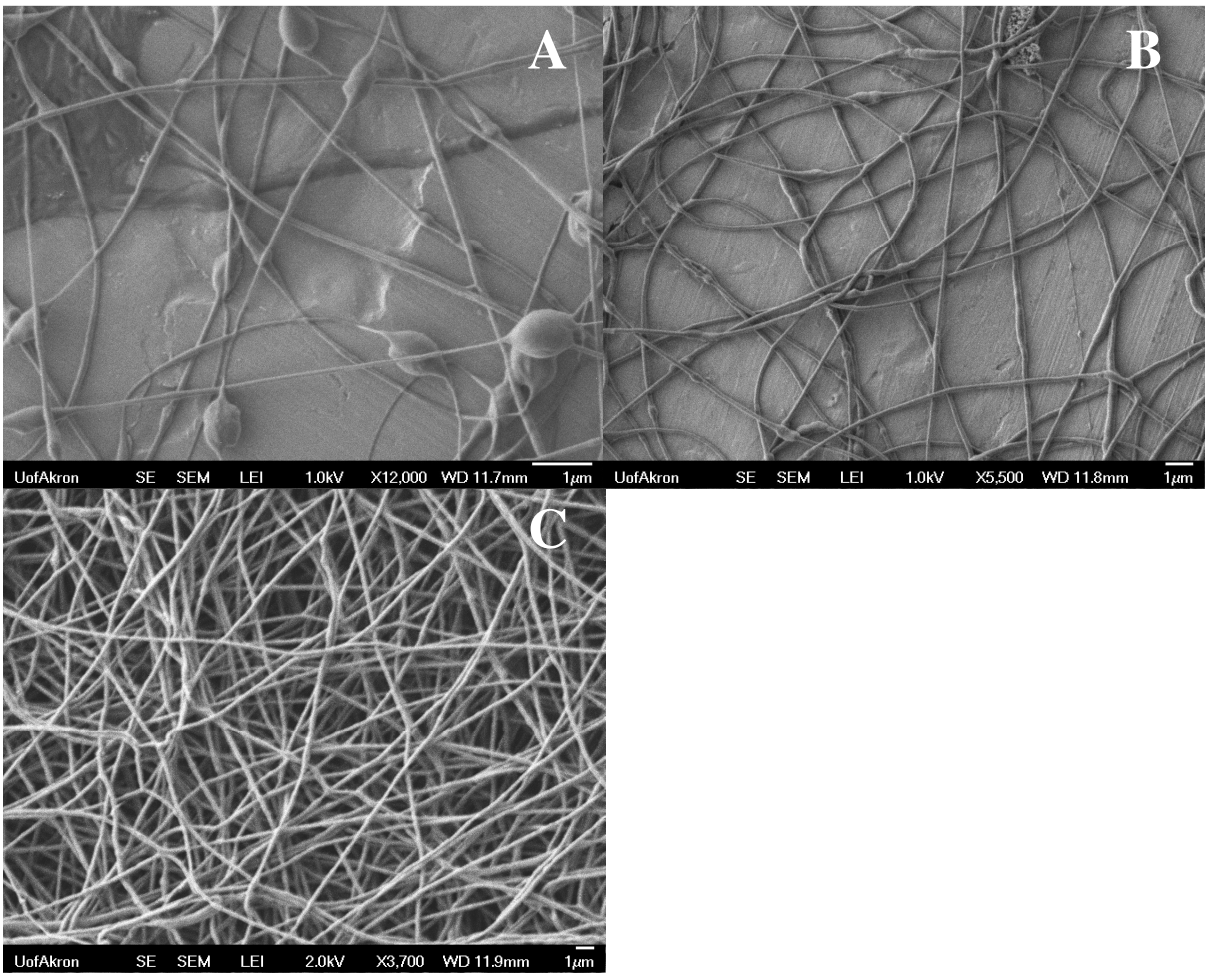

Figure 8. A. 4% PEO at 15 cm, 20 kV, and 20 μL/min. B. 5% PEO at 15 cm, 20 kV, and 20 μL/min. C. 6% PEO at 15 cm, 20 kV, and 20 μL/min.

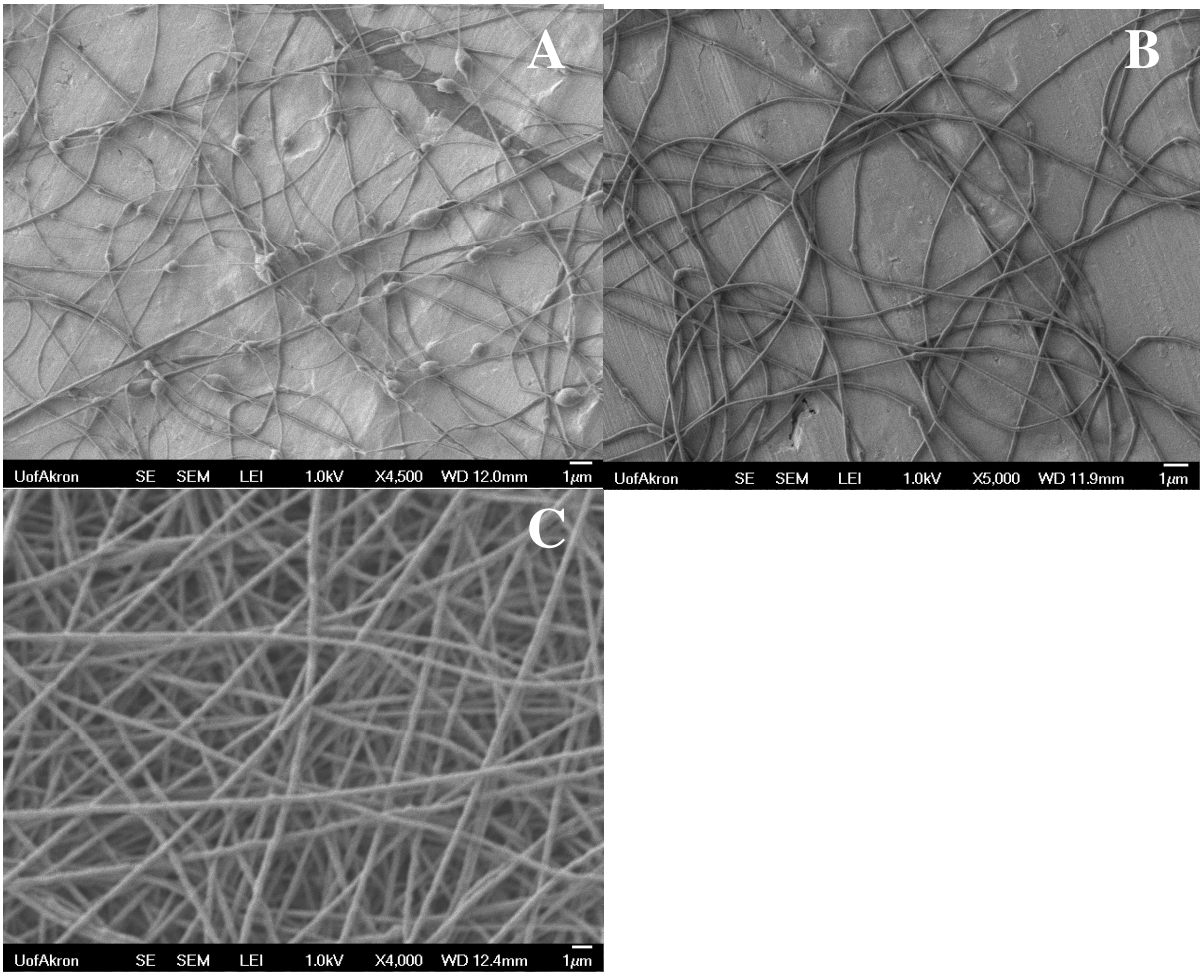

Figure 9. A. 4% PEO at 15 cm, 25 kV, and 20 μL/min. B. 5% PEO at 15 cm, 25 kV, and 20 μL/min. C. 6% PEO at 15 cm, 25 kV, and 20 μL/min.

The surface tension of PEO and PEO and Crocin mixtures was analyzed to determine the effect of the Crocin on the PEO solution. **Figure 10** shows the surface tension results for 4%, 5%, and 6% PEO mixed with 0.24% and 1% Crocin. Lower amounts of Crocin resulted in a higher surface tension compared to higher amounts of Crocin in the solution. Adding Crocin to the PEO solution resulted in a lower surface tension in all but the 6% PEO mixed with 0.24% Crocin.

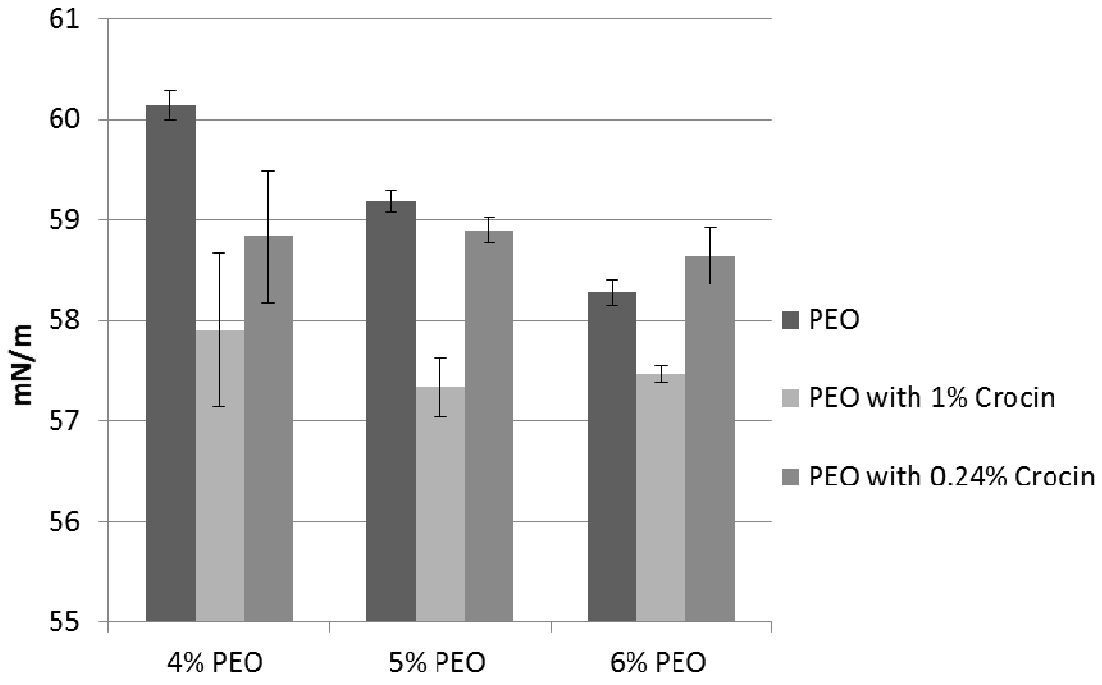

Figure 10. Surface tension of various concentrations of PEO mixed with Crocin.

The viscosity at shear rates of 5, 10, 20, 50, and 100 RPM for 4%, 5%, and 6% PEO was analyzed as seen in **Figure 11**. Viscosity of the PEO with 0.24% Crocin and 1% Crocin was also analyzed as seen in **Figure 12** and **Figure 13**. Without Crocin, the PEO solutions had a higher viscosity, with 5% having the highest followed by 6% and 4%. At higher shear rates, the viscosity was lower than at lower shear rates. There is little to no difference between the viscosities of the 0.24% Crocin and 1% Crocin solutions.

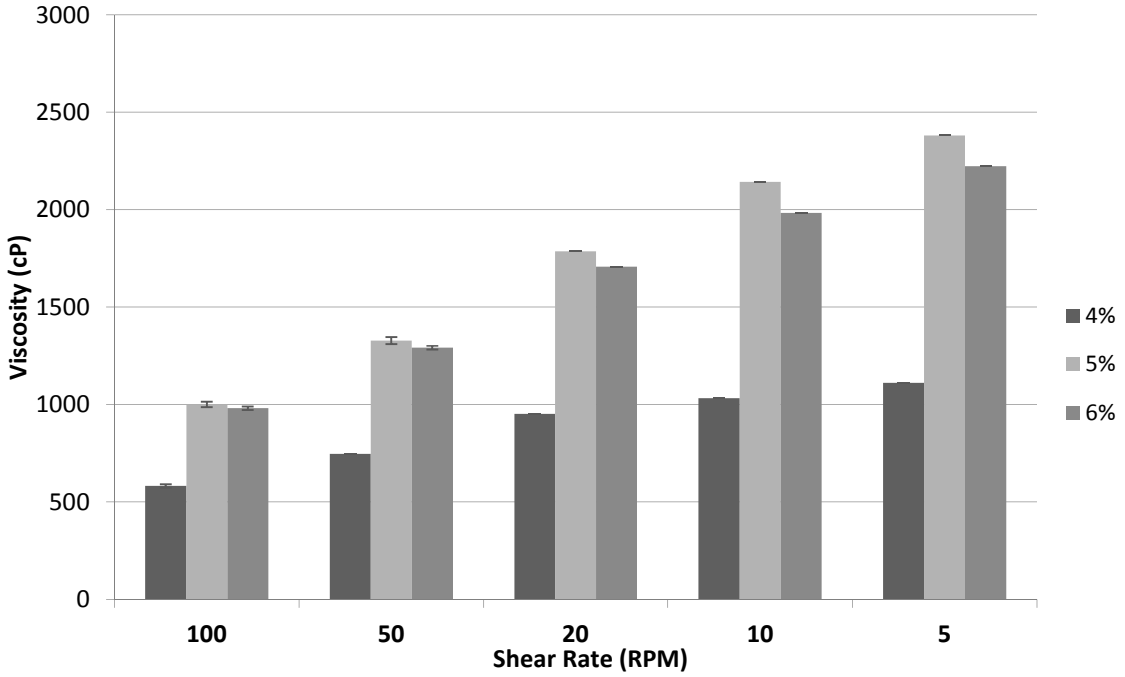

Figure 11. Viscosity of varying PEO concentrations at varying shear rates.

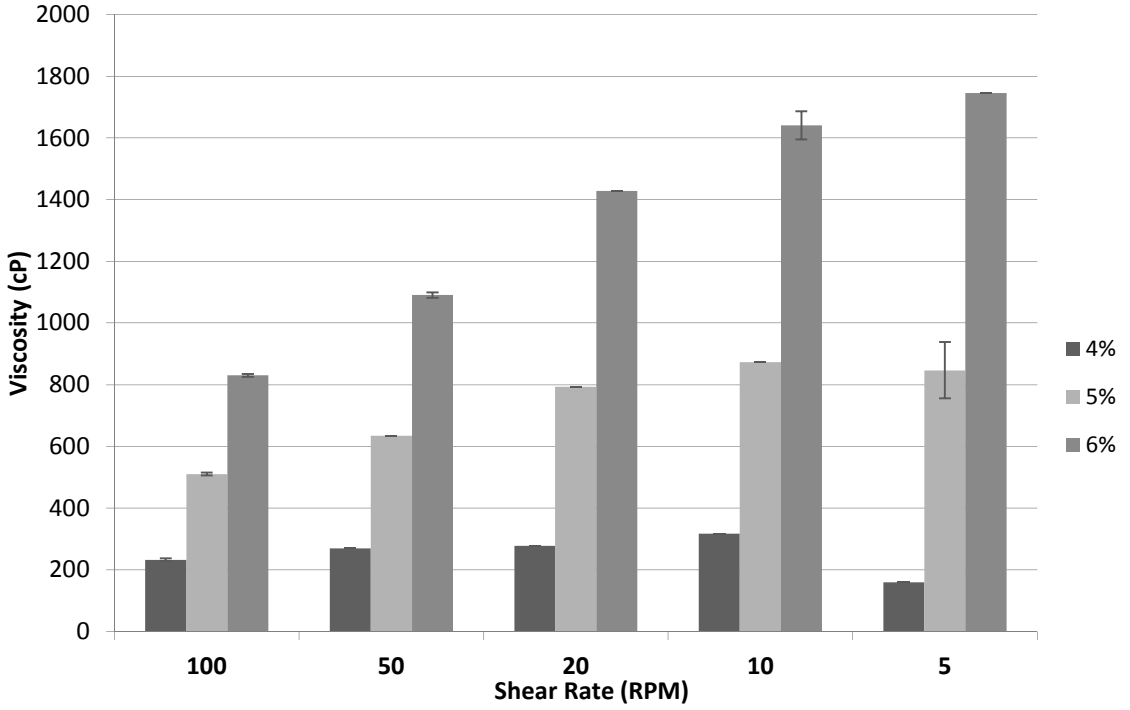

Figure 12. Viscosity of varying concentrations of PEO with 0.24% Crocin at varying shear rates.

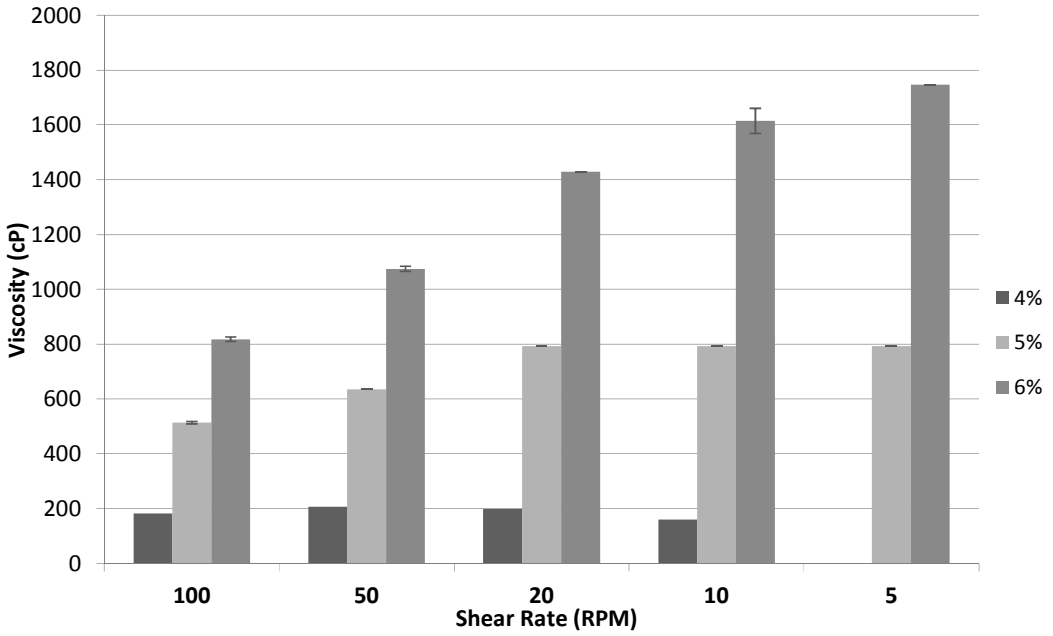

Figure 13. Viscosity of varying concentrations of PEO with 1% Crocin at varying shear rates.

Using the above information, 6% PEO with 1% Crocin was spun at two conditions: 20 cm, 40 µL/min, and 20 kV and 20 cm, 60 µL/min, and 25 kV. Fiber diameters are shown in **Figure 14**. Both conditions had a range of fiber diameters as indicated by the error bars which represent one standard deviation. The fiber diameter of the 6% PEO spun at 40 µL/min, 20 kV, and 20 cm is 0.46±0.06 microns. The fiber diameter of the 6% PEO with 1% Crocin spun at 40 µL/min, 20 kV, and 20 cm is 0.32±0.04 microns. The fiber diameter of the 6% PEO spun at 60 µL/min, 25 kV, and 20 cm is 0.45±0.06 microns. The fiber diameter of the 6% PEO with 1% Crocin spun at 60 µL/min, 25 kV, and 20 cm is 0.29±0.05 microns. The addition of Crocin resulted in a smaller fiber diameter than PEO without Crocin. **Figure 15** show example images of the measured fibers from **Figure 14**. The addition of Crocin resulted in smoother fibers than the PEO electrospun at the same conditions.

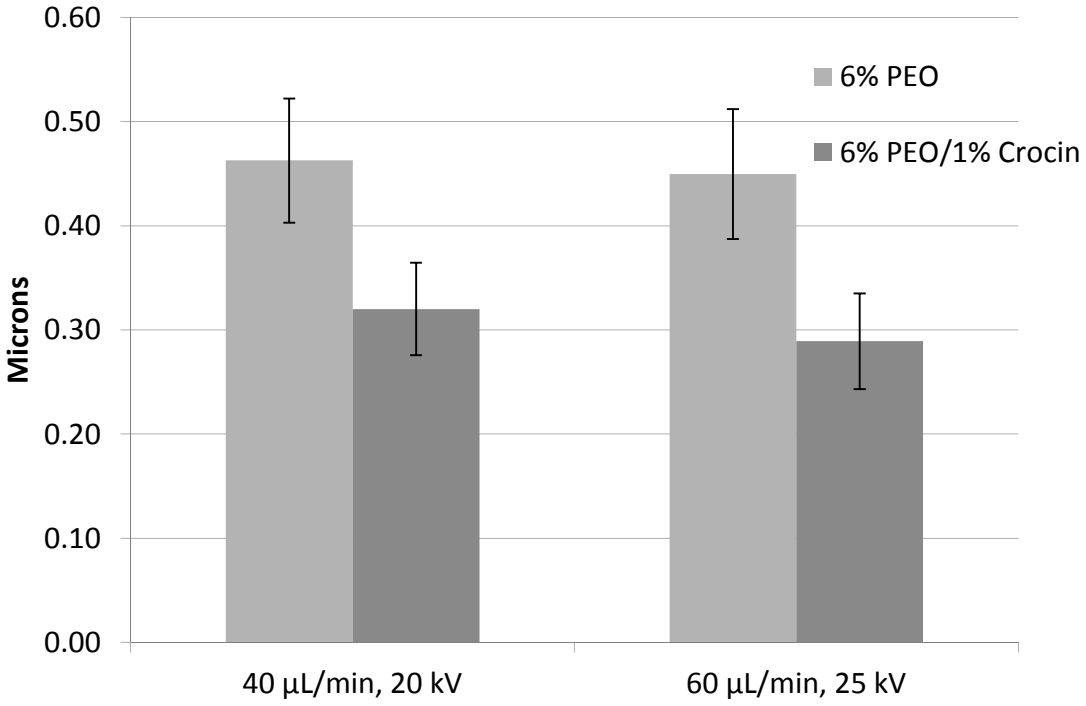

Figure 14. Fiber diameter of 6% PEO and 6% PEO with 1% Crocin at a constant 20 cm and 40 µL/min and 20 kV and 60 µL/min and 25 kV.

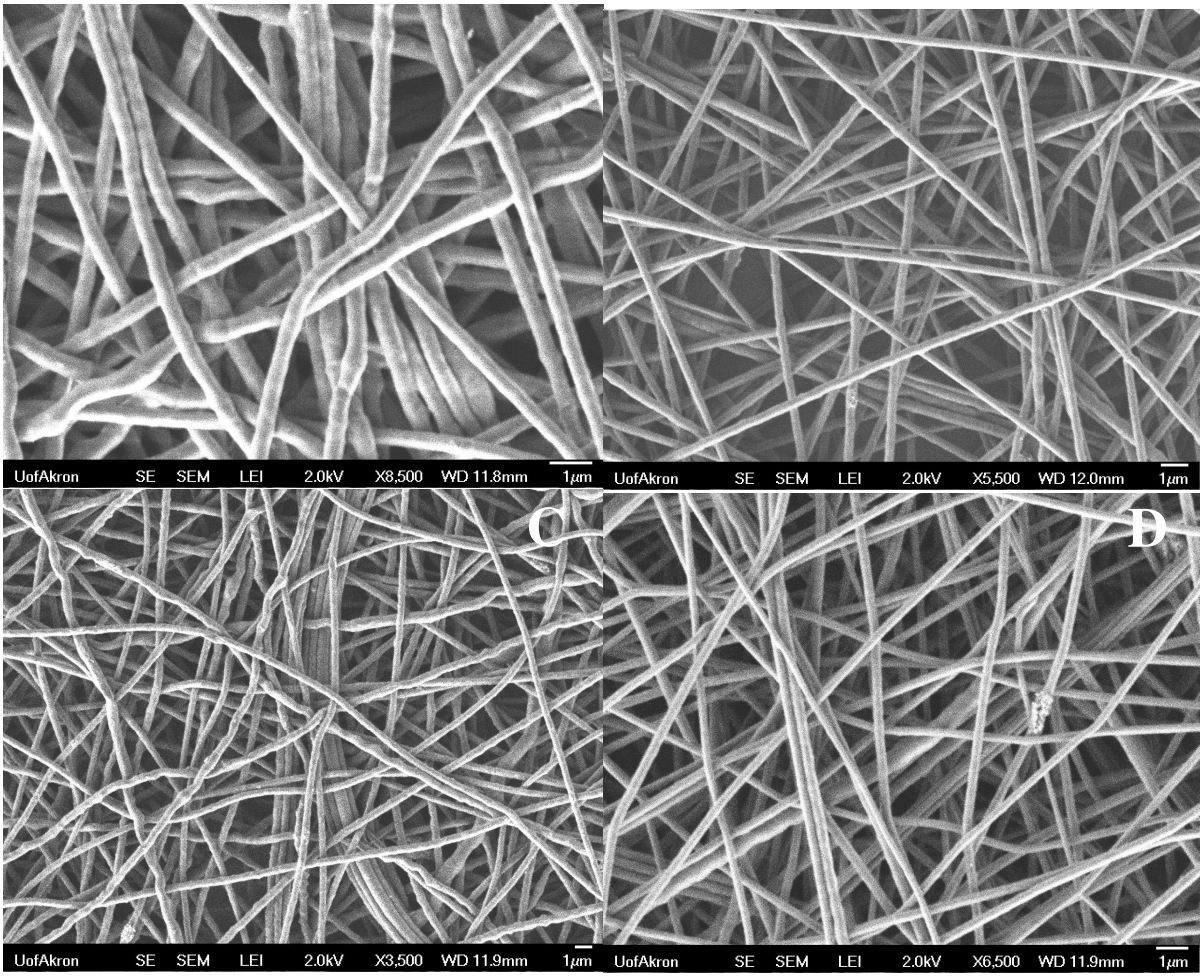

Figure 15. A. 6% PEO at 20 cm, 40 μL/min, and 20 kV. B. 6% PEO with 1% Crocin at 20 cm, 40 μL/min, and 20 kV. C. 6% PEO at 20 cm, 60 μL/min, and 25 kV. D. 6% PEO with 1% Crocin at 20 cm, 60 μL/min, and 25 kV.

Discussion and Analysis

The PEO study followed the expected results. Increasing voltage can lead to a decrease in fiber diameter initially, but during further increase, the diameter can increase. This can be seen in **Figure 6** where the fiber diameter of the 5% PEO increased with increasing voltage and with the 6% PEO where the fiber diameter initially decreased at 20 kV, but fiber diameter increased at 25 kV. Lower voltages can also cause increased bead defects, which can be seen in **Figure 7**, **Figure 8**, and **Figure 9** where the lower voltages for the 5% have increased bead defects and the 6% fibers become smoother with increased voltages. Fibers were not able to be collected at the lower flow rates most likely due to an inability to maintain the Taylor cone at the tip of the needle. Too high of flow rate led to inadequate drying time resulting in very wet fibers that clumped and flattened into ribbons. The low gap distance also led to wet fibers that clumped and formed flattened ribbons.

The surface tension and viscosity also followed the expected results. The addition of Crocin lowered the surface tension of the solutions. This follows the expected result since surfactants lower the surface tension. The viscosity of the PEO and Crocin solutions were also lower than the PEO solutions. The solutions exhibited a shear thinning behavior where at higher shear rates the viscosity of the solution was lower than at lower shear rates.

The addition of Crocin was supposed to improve the PEO fiber diameter by making the diameter smaller and improve the fiber morphology by reducing the surface tension of the solution. The added Crocin accomplished both of these. The fiber diameter was reduced by 31%±1% for the sample spun at 20 kV and 37%±0.5% for the sample spun at 25 kV. Also the fiber morphology improved. In **Figure 15**, as seen in A and C, the fibers are not as smooth as the fibers in B and D. The Crocin helped to create smoother fibers and smaller fibers which would have a larger surface area to volume ratio.

Next steps would include a more expansive study of the PEO and Crocin role which would include more electrospinning characterization and looking into whether the Crocin is located in or on the fiber surface. If the Crocin is on the fiber surface, the Crocin may be able to be modified to change the surface chemistry of the fiber for drug loading or other applications.

Acknowledgements

I would like to thank Rebecca Chase, the graduate student who helped me with the research, design, and experiments. She was always willing to help and answer any questions I had or direct me to someone who could help. I would also like to thank Dr. Puskas for sponsoring the project and her input into the research and report.

Bibliography

Kriegel, C.; Kit, K.M.; McClemenets, D.J.; Weiss, J. Influence of Srfactant Type and Concentration on Electrospinning of Chitosan-Poly(Ethylene Oxide) Blend Nanofibers. *Food Biophysics*. [Online] **2009**, 4, 213-228. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Li, Dan and Xia, Younan. Electrospinning of Nanofibers: Reinventing the Wheel? *Advanced Materials*. [Online] **2004**, 16, 1151-1170. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Naess, Stine Nalum; Elgsaeter, Arnljot; Foss, Bente Jeanette; Li, Baojun; Sliwka, Hans-Richard; Partali, Vassilia; Melø, Thor Bernt; Naqvi, K. Razi. Hydrophilic Carotenoids: Surface Properties and Aggregation of Crocin as a Biosurfactant. *Helvetica Chimica Acta*. **2006**, 89, 45-53.

Nagiah, Naveen; Sivagnanam, Uma Tiruchirapalli; Mohan, Ranganathan; Srinivasan, Natarajan Tirupattur; Sehgal, Praveen Kumar. Development and Characterization of Electrospun Poly(propylene carbonate) Ultrathin Fibers as Tissue Engineering Scaffolds. [Online] **2012**, 14, 4. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Sill, Travis; von Recum, Horst. Electrospinning: Applications in drug delivery and tissue engineering. *Biomaterials*. [Online] **2008**, 29, 1989-2006. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Subbiah, Thandavamoorthy; Bhat, G.S.; Tock, R.W.; Parameswaran, S.; Ramkumar, S. Electrospinning of Nanofibers. *Journal of Applied Polymer Science*. [Online] **2005**, 96, 557-569. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Tamaddonfard, Esmaeal and Hamzeh-Gooshchi, Nasrin. Effect of Intraperitoneal and Intracerebroventricular Injection of Crocin on Acute Corneal Pain in Rats. *Phytotherapy Research*. [Online] **2010**, 24, 1463-1467. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Wang, Shu-Qiang; He, Ji-Huan; Xu, Lan. Non-ionic surfactants for enhancing electrospinability and for the preparation of electrospun nanofibers. *Polymer International*. [Online] **2008**, 57, 1079-1082. SciFinder. http://www.scifinder.com (accessed Nov 2012).

Ziani, Khalid; Henrist, Catherine; Jérôme; Aqil, Abdelhafid; Maté, Juan; Cloots, Rudi. Effect of nonionic surfactant and acidity of chitosan nanofibers with different molecular weights. *Carbohydrate Polymers*. [Online] **2011**, 83, 470-476. SciFinder. http://www.scifinder.com (accessed Nov 2012).